

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of:
Helke Hillebrand et al.

Application No.: 10/593,181

Confirmation No.: 6326

Filed: September 15, 2006

Art Unit: 1638

For: IMPROVED CONSTRUCTS FOR MARKER
EXCISION BASED ON DUAL-FUNCTION
SELECTION MARKER

Examiner: C. K. Worley

BRIEF ON APPEAL

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APPEAL BRIEF UNDER 37 C.F.R. § 41.37

MS Appeal Brief - Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Applicants hereby appeal the Examiner's decision rejecting claims 1-4, 10, and 27 as set forth in the Office Action of December 2, 2009.

As required under 37 C.F.R. § 41.37(a), this brief is filed within two months of the filing of the Notice of Appeal, which was filed on April 1, 2010, with the required fee authorization pursuant to § 41.20(b)(2).

This brief contains items under the following headings as required by 37 C.F.R. § 41.37 and M.P.E.P. § 1205.2:

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I. REAL PARTY IN INTEREST

The real parties in interest are BASF Plant Science GmbH and SweTree Technologies AB (“BASF”), the assignees of record.

II. RELATED APPEALS, INTERFERENCES, AND JUDICIAL PROCEEDINGS

There are no other appeals, interferences, or judicial proceedings which will directly affect or be directly affected by or have a bearing on the Board’s decision in this appeal.

III. STATUS OF CLAIMS

A. Total Number of Claims in Application

There are 30 claims pending in application.

B. Current Status of Claims

Claims cancelled: none

Claims withdrawn from consideration but not cancelled: 5-9, 11-26, and 28-30

Claims pending: 1-30

Claims allowed: none

Claims rejected: 1-4, 10, and 27

Claims objected to: none

C. Claims On Appeal

The claims on appeal are claims 1-4, 10, and 27

A copy of the appealed claims as they currently stand is provided in Section VIII as Appendix A.

IV. STATUS OF AMENDMENTS

A Final Office Action was mailed December 2, 2009 in which the rejections under 35 U.S.C. § 112, first paragraph, and 35 U.S.C. § 103(a) remained. In response, Applicants filed an Amendment And Reply Under 37 CFR §1.116 on March 8, 2010. The Examiner responded to the Amendment And Reply Under 37 CFR §1.116 in an Advisory Action dated March 18, 2010.

In the Advisory Action, the Examiner indicated that Applicants' amendments to the claims would be entered. Accordingly, the claims enclosed herein as Appendix A incorporate the amendments of the Amendment And Reply Under 37 CFR §1.116 of March 8, 2010.

V. SUMMARY OF CLAIMED SUBJECT MATTER

Of the six claims on appeal, claim 1 is the only independent claim on appeal and reads as follows (with appropriate cross-reference to the specification in parenthesis):

1. A method for producing a transgenic plant (p. 5, ll. 23-24; original claim 1) comprising:
 - i) transforming a plant cell with a first expression cassette comprising a nucleic acid sequence encoding a D-amino acid oxidase (p. 30, ll. 39-42; p. 32 l. 29 through p. 38 l. 39; p. 83) operably linked with a promoter allowing expression in plant cells or plants, in combination with at least one second expression cassette suitable for conferring to said plant an agronomically valuable trait (p. 5, ll. 26-30; p. 12, ll. 20-38; Figures 1, 8-10; original claim 1), and
 - ii) providing at least one first compound X, which is phytotoxic against plant cells not functionally expressing said D-amino acid oxidase, wherein said compound X can be metabolized by said D-amino acid oxidase into one or more compound(s) Y which are non-phytotoxic or less phytotoxic than compound X (p. 5, ll. 32-35; p. 7, ll. 28-32; p. 39, ll. 2-30; p. 46, ll. 2-34; Example 5; original claim 1), and
 - iii) treating said transformed plant cells of step i) with said first compound X in a phytotoxic concentration and selecting plant cells comprising in their genome both said first and said second expression cassette, wherein said first expression cassette is conferring resistance to said transformed plant cells against said compound X by expression of said D-amino acid oxidase (p. 5, ll. 37-41; p. 7, ll. 28-32; p. 39, ll. 2-30; p. 46, ll. 2-34; Example 5; original claim 1), and
 - iv) providing at least one second compound M, which is non-phytotoxic or moderately phytotoxic against plant cells not functionally expressing said D-amino acid oxidase, wherein said compound M can be metabolized by said D-amino acid oxidase into one or more compound(s) N which are phytotoxic or more phytotoxic than compound M (p. 6, ll. 1-5; p. 7, ll. 34-39; p. 39 l. 32 through p. 40 l. 23; p. 46 l. 37 through p. 47 l. 18; Example 5; original claim 1), and

- v) breaking the combination between said first expression cassette and said second expression cassette and treating resulting said plant cells with said second compound M in a concentration toxic to plant cells still comprising said first expression cassette, and selecting plant cells comprising said second expression cassette but lacking said first expression cassette (p. 6, ll. 7-11; p. 29, ll. 4-36; Example 5; original claim 1),

wherein said first compound X and said second compound M both comprise a D-amino acid (p. 7, ll. 28-39; p. 31, ll. 22-26, 35-40; Example 5).

VI. GROUND OF REJECTION TO BE REVIEWED ON APPEAL

In the Advisory Action dated March 18, 2010, the written description rejection under 35 U.S.C. § 112 of the Final Office Action dated December 2, 2009, was withdrawn. Accordingly, this rejection is not addressed in this Appeal.

The grounds of rejection for review on appeal are as follows:

Whether claims 1-4, 10, and 27 are unpatentable under 35 U.S.C. § 103(a) as being obvious over Signer *et al.* WO 01/96583 (“Signer”) in view of Nasholm *et al.* WO 03/060133 (‘Nasholm’) “and taken with the evidence of Stougaard” *et al.* (‘Stougaard’) “and the evidence of Boeke *et al.*” (‘Boeke’)? (see Office Action dated December 2, 2009, p. 8; Advisory Action dated March 18, 2010, p. 2)

VII. ARGUMENT

Claims 1-4, 10, and 27 are not obvious under 35 U.S.C. § 103(a) over Signer *et al.* WO 01/96583 (‘Signer’) in view of Nasholm *et al.* WO 03/060133 (‘Nasholm’) and taken with the evidence of Stougaard *et al.* (‘Stougaard’) and the evidence of Boeke *et al.* (‘Boeke’).

A. Overview of the References Cited

- a) The Differences Between The Claimed Subject Matter And The Primary Reference Signer.

Signer teaches a method for removing selectable marker genes from transformed plant cells utilizing two different selectable markers, *i.e.* a positive selectable marker and a different, negative selectable marker. (Signer, page 2, paragraph [0007], Examples 1-3, claims 1-3, 10-16,

Figure 1). Signer provides the following general formula to depict the construct used in their method: GI-PS-NS-GI or GI-NS-PS-GI, where GI is the gene of interest, PS is the positive selectable marker gene, and NS is the negative selectable marker gene. (Signer, p. 2, ¶¶ [0006]-[0007]). The construct taught in Signer uses an NPT gene as the positive selectable marker and the *CodA* gene as the negative selectable marker (Signer, pp. 13-16, Examples 1-3). Signer further discloses that the positive selection medium comprises kanamycin which acts upon the positive selectable marker, *i.e.* the NPT gene, and that the negative selection medium comprises 5-fluorocytosine, which acts with the negative selectable marker, *i.e.* the *CodA* gene (Signer, for example, pp. 13-16, Examples 1-3).

Signer's specific method steps thus require using a positive selection medium which specifically acts with the positive selectable marker (*i.e.* the NPT gene) and a **separate** negative selection medium which specifically acts with the negative selectable marker (*i.e.* the *CodA* gene). (Signer, p. 14). By contrast claims 1-4, 10 and 27 recite a method using only one selectable marker as a **dual-functional selection marker**, *i.e.* one gene as both a positive and a negative selectable marker, for eliminating marker sequences from plant cells rather than two separate selectable marker genes as taught by Signer. Additionally by contrast the claims recite that each of the selection media act on the same selectable marker, rather than the two selection media acting with two different types of selectable markers as taught by Signer. Nothing in Signer teaches that the positive selection media and the negative selection media could act with only one selectable marker or on the same selectable marker.

Furthermore, the construct taught in Signer also comprises direct repeats of the gene of interest (e.g. valuable trait) which flank the two different types of selectable markers. (Signer, p. 2, ¶ [0006]). Signer discloses that during vegetative growth and meiosis, intrachromosomal homologous recombination (IHR) between the direct repeats of the gene of interest in the construct promotes crossing-over that loops out and eliminates the intervening DNA (*i.e.* the DNA between the direct repeats of the gene of interest), leaving behind a construct with only a single copy of the gene of interest itself (Signer, pp. 2, 5, 13; Examples 1-3). Signer teaches that direct repeats of the gene of interest are required in the particular configuration in the construct in order to allow for homologous recombination, crossing over, and the elimination of the selectable markers (Signer, pp. 2, 5, 13; Examples 1-3). Thus, in Signer, the direct repeats of the

gene of interest themselves correspond to the sequences which allow for the elimination of the selectable markers. By contrast, claims 3, 10, and 27 recite that the first expression cassette which comprises the nucleic acid sequence encoding a D-amino acid oxidase (*i.e.* the selectable marker) is flanked by sequences which allow for specific deletion of the first expression cassette, but that the second expression cassette, which is suitable for conferring an agronomically valuable trait, is not localized between the sequences which allow for the specific deletion of the first expression cassette.

b) Brief Summary of the Cited Secondary References.

The Examiner acknowledges that the construct of Signer is different than the construct used in the claimed method (Office Action dated December 2, 2009, p. 9). The Examiner also acknowledges that Signer does not teach or suggest the claimed sequence encoding a D-amino acid oxidase or the use of a D-amino acid oxidase gene as both a positive and a negative selectable marker (Office Action dated December 2, 2009, p. 9). Nasholm is relied on for teaching D-amino acid oxidase and that D-amino acid oxidase can be used as either a negative selection marker or a positive selection marker (Office Action dated December 2, 2009, p. 9). Stougaard is relied on for teaching that *CodA* can be used as either a negative selection marker or a positive selection marker (Office Action dated December 2, 2009, p. 10). Finally, Boeke is relied on for allegedly teaching that one selectable marker can be used as both a positive and a negative selectable marker (Office Action dated December 2, 2009, pp. 10, 12). However, none of the teachings of the secondary references remedy the deficiencies of the primary reference Signer.

Nasholm relates to genes encoding enzymes that metabolize D-amino acids and plants expressing such genes in order to utilize D-amino acids as a nitrogen source and grow on media which would not otherwise support growth of the wild-type plant. (Nasholm, p. 2, ll. 24-28). Nasholm also relates to D-amino acids that may be used for selection by transforming a plant with a nucleic acid expressing a D-amino acid metabolizing protein such as D-amino acid oxidase. Nasholm does not teach or disclose the use of D-amino acid oxidase as a dual-functional selection marker for eliminating selectable marker sequences from transgenic plants.

Stougaard discloses that *CodA* can be used either as a negative selectable marker or a positive selectable marker. Nothing in Stougaard discloses the use of *CodA* as a dual-functional

selection marker in one construct.

The entire premise of the Examiner's obviousness argument revolves on Boeke allegedly teaching one transgene being used as a dual-functional selectable marker for first identifying transformants and second for excising the marker. (Office Action dated December 2, 2009, pp. 10, 12). However, the disclosure of Boeke does not support this supposition.

Boeke describes using the *URA3* gene in a yeast system (Boeke, pp.164, 166, Fig. 1). The transformed yeast cells comprising the *URA3* gene disclosed in Boeke are first selected for the Ura⁺ phenotype (Boeke, p. 166), which entails selection based on a medium lacking uracil (See Office Action dated December 2, 2009, p. 10, ll. 15-17). The transformed cells which are capable of producing uracil (*i.e.* Ura⁺) grow on this medium, whereas the wild-type cells which require uracil for growth are basically starved because of the absence of uracil in the media and thus cannot grow.

Boeke further discloses that "selective techniques usually depend on the conversion of a nontoxic compound to one that is toxic to wild-type cells." (Boeke, p. 164, first ¶). Boeke teaches that in yeast only a few selective compounds of this type have been discovered, one of which is 5-fluoroorotic acid (5-FOA). (Boeke, p. 164, first ¶).

Boeke teaches using 5-FOA for selecting yeast cells which have lost the *URA3* gene and the flanking plasmid DNA *via* a homologous recombination event (Boeke, pp. 164, 166, Fig. 1). Cells which have lost the *URA3* gene, referred to as Ura⁻, can survive on medium containing 5-FOA, whereas the cells containing the *URA3* gene cannot, based on the conversion by the cell containing the *URA3* gene of the nontoxic precursor compound to one that is toxic (Boeke, p. 164, first and second ¶¶). Boeke thus teaches that their system only uses one selection medium comprising one compound (*i.e.* the 5-FOA) that actually acts with the selectable marker.

Boeke does not teach or disclose a first selection medium containing a compound which is toxic to the wild-type cell and which is metabolized by the marker in the transformed cell into a non-toxic or less toxic compound as in claim 1 steps ii) and iii). Rather Boeke only teaches a single selection where a nontoxic compound is converted to one that is toxic (Boeke, p. 164, first paragraph). The initial selection of Ura⁺ transformants of Boeke does not teach use of a toxic compound corresponding to compound X as claimed and as such does not relate to a selectable

marker acting with a compound for selection. In Boeke's initial selection, the selection is passive with the marker not being involved in any reaction with components of the media for causing the selection. Thus Boeke like the other secondary references does not teach the dual-functional selectable marker of the claims on appeal.

B. The Claims Are Not Obvious To A Person Of Ordinary Skill In The Art From The Reading Of Signer, Nasholm, Stougaard, And Boeke

a) The Examiner's Combination of References Is Misplaced.

The principal flaw in the Examiner's rejection is the absence of teaching of a dual-functional selection marker for excising the marker using two different compounds capable of acting on the one dual-functional marker.

The Examiner bears the initial burden of establishing *prima facie* obviousness. See *In re Rijckaert*, 9 F.3d 1531, 1532, 28 USPQ2d 1955, 1956 (Fed. Cir. 1993). To support a *prima facie* conclusion of obviousness, the prior art must disclose or suggest all the limitations of the claimed invention. See *In re Lowry*, 32 F.3d 1579, 1582, 32 USPQ2d 1031, 1034 (Fed. Cir. 1994); see also *Abbott Labs. v. Sandoz, Inc.*, 544 F.3d 1341 (Fed. Cir. 2008) ("[t]he KSR opinion ... did not mention or affect the requirement that each and every claim limitation be found present in the combination of the prior art references before the analysis proceeds." (emphasis added) (quoting *Abbott Labs. v. Sandoz, Inc.*, 500 F.Supp.2d 846, 852 (N.D.Ill. 2007))).

The Examiner contends that Signer teaches a method of generating a transgenic plant that utilizes both a positive and a negative selection marker in order to remove the selection markers from the resulting transgenic plants. (Office Action dated July 28, 2009, p. 11). However, as noted above (supra p.), the construct of Signer requires **two** different selectable markers (Signer, p. 2, ¶ [0007], Examples 1-3, claims 1-3, 10-16, Fig. 1) rather than the recited **single** dual-functional marker. Furthermore, the positive selection medium used in Signer acts with the NPT gene, the positive selectable marker, and the negative selection medium acts with the *CodA* gene, the negative selectable marker. Thus, the **two** selection media act with **two** different types of selectable markers.

In contrast to Signer, the present claims relate to the use of only **one** enzyme, D-amino acid oxidase, for both positive and negative selection in one construct as a dual-functional

marker for eliminating marker sequences from transgenic plants. Further in contrast to Signer, the **two** selection compounds of the present claims (compound X and M) act with only **one** selectable marker (D-amino acid oxidase).

As explained above, the Examiner acknowledges that Signer does not teach the claimed sequence encoding a D-amino oxidase or its use as both a positive and a negative selectable marker. Therefore, the Examiner falls back on the teaching of Nasholm. Nasholm however does not teach or disclose the use of D-amino acid oxidase as a dual-functional selection marker for eliminating marker sequences from transgenic plants.

The Examiner nevertheless contends that one skilled in the art would surmise that “one would only require one transgene rather than two separate selectable marker genes.” (Final Office Action dated December 2, 2009, p. 10). The only cited support for this supposition is an assertion by the Examiner that “[t]his concept was generally known in the art,” citing to Stougaard and Boeke (Final Office Action dated December 2, 2009, p. 10).

However, the disclosure of Stougaard does not support this supposition. Stougaard discloses that *CodA* can be used either as a negative selectable marker or as a positive selectable marker. (Stougaard, abstract, p. 759 left col., p. 760 left col.). However, even with this knowledge, Signer still teaches that two distinct selectable markers are required for the production of marker-free plants even if one of the selectable markers used in the Signer construct was *CodA*, a potential dual functional marker.

Thus the entire premise of the Examiner’s obviousness argument rests on Boeke allegedly teaching one transgene being used as a dual-functional selectable marker for first identifying transformants and second for excising the marker. (Office Action dated December 2, 2009, pp. 10, 12). However, the disclosure of Boeke does not support this supposition.

As explained above, the selection method taught by Boeke relates to yeast cells with an initial phenotypic selection conducted by simply not providing the necessary elements for growth in the medium (*i.e.* lacking uracil). This medium does not require a compound that acts on the selectable marker, *i.e.* a compound that is toxic to wild-type cells and which is converted to a non-toxic compound by the transgene. Boeke teaches that their system only uses one selection medium comprising one compound (*i.e.* the 5-FOA) that actually acts with the selectable marker,

i.e. the *URA3* gene. Boeke thus teaches one transgene as a single selection marker using only one compound being converted. Thus, the yeast system of Boeke is totally different than the methods described in plants such as using the *CodA* gene described in Stougaard, the D-amino acid oxidase of Nasholm, or the method of Signer which uses two selection media acting with two different types of selectable markers (the *CodA* gene and the NPT gene) and as such the references are not combinable.

Even assuming *arguendo* that one skilled in the art would consider combining these four references, they do not disclose the use of a single selectable marker such as D-amino acid oxidase as a dual-functional selection marker for eliminating marker sequences from transgenic plants with the use of compounds such as compounds X and M as claimed. Moreover, because Boeke does not teach one transgene being used as a dual-functional selectable marker, the entire obviousness argument falls apart.

Because the combined teaching of the references cited by the Examiner does not disclose or suggest all the claim limitations, a *prima facie* case of obviousness has not been established. For this reason alone, the obviousness rejection should be reversed.

b) The Modification Suggested By The Examiner Requires A Substantial Reconstruction And Redesign Of The Construct Being Modified Thereby Changing The Basic Principle Under Which The Method Being Modified Operates.

If the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims *prima facie* obvious. *In re Ratti*, 270 F.2d 810, 123 USPQ 349 (CCPA 1959) (The court reversed the obviousness rejection holding the “suggested combination of references would require a substantial reconstruction and redesign of the elements shown in [the primary reference] as well as a change in the basic principle under which the [primary reference] construction was designed to operate.” 270 F.2d at 813, 123 USPQ at 352.). MPEP § 2143.01 VI.

The Examiner’s proposed combination of the cited references fatally alters their methods.

i) Signer modified by Nasholm.

The obviousness conclusion expressed in the Final Rejection of December 2, 2009, states that substituting the construct taught by Nasholm for the construct utilized by Signer does not require a substantial reconstruction of the method or principles taught by Signer (Final Office Action dated December 2, 2009, p. 15, ll. 12-14). Appellants strongly disagree.

Assuming *arguendo*, Signer and Nasholm were combinable, the proposed modification to Signer suggested by the Examiner does require a “substantial reconstruction and redesign” of elements and changes the principle under which Signer operates.

Signer describes using a construct comprising direct repeats of the gene of interest which flank at least two different types of selectable markers. (Signer, p. 2, ¶ [0006]). Signer provides the following general formula to depict the construct: GI-PS-NS-GI or GI-NS-PS-GI, where GI is the gene of interest, PS is the positive selectable marker gene, and NS is the negative selectable marker gene. (Signer, p. 2, ¶¶ [0006]-[0007]). Signer also discloses the positive selectable marker as a NPT gene and the negative selectable marker as the *CodA* gene (Signer pp. 13-16, Examples 1-3). Signer further discloses that the positive selection medium comprises kanamycin which acts upon the positive selectable marker, *i.e.* the NPT gene, and that the negative selection medium comprises 5-fluorocytosine (5-FOA), which acts with the negative selectable marker, *i.e.* the *CodA* gene (Signer, for example, pp. 13-16, Examples 1-3).

The Examiner also asserts that Signer “was relied upon to teach the specific method steps.” (Final Office Action dated December 2, 2009, p. 15, l. 8). The specific method steps described by Signer require **two** different types of selectable markers and **two** different selection media comprising compounds where each of the compounds acts with a different selectable marker. Signer does not teach that each of the selection media could act on the same selectable marker. Nothing in Signer teaches that the positive selection media and the negative selection media could act on the same selectable marker.

The modification suggested by the Examiner is to substitute the construct taught by Nasholm for the construct utilized by Signer in the method taught by Signer (Final Office Action dated December 2, 2009, pp. 12-14).

Assuming *arguendo* the constructs were substitutable, there are two options for effectuating this modification.

In the first option, the redesign would require at least four quantum leaps between the construct of Signer and the claims:

- 1) eliminating both the negative selectable marker and the positive selectable marker, *i.e.* the NPT gene and the *CodA* gene;
- 2) replacing these two genes with a construct with one single selectable marker gene which could be used as both a positive and a negative selection marker;
- 3) eliminating the positive selection medium that comprises kanamycin which had previously acted on the positive selectable marker, *i.e.* the NPT gene, and replacing it with one that could act on the newly inserted marker for positive selection; and
- 4) eliminating the negative selection medium that comprises 5-FOA which had previously acted on a separate distinct marker, the negative selectable marker *CodA*, and replacing it with one that could also act on the one newly inserted marker for negative selection.

In the second option, the redesign would require at least five quantum leaps between the construct of Signer and the claims:

- 1) eliminating the positive selectable marker, *i.e.* the NPT gene, leaving the *CodA* gene as the one single selectable marker known as a marker which could be used either as a positive or negative selection marker (based on the teaching of Stougaard);
- 2) substituting *CodA* with D-amino acid oxidase;
- 3) eliminating two selection media which were designed to each act on separate different selectable markers;
- 4) replacing the positive selection medium that comprises kanamycin which had previously acted on the positive selectable marker, *i.e.* the NPT gene, with one that could act on D-amino acid oxidase for positive selection;
- 5) replacing the negative selection medium that comprises 5-FOA which had previously acted on a separate distinct marker, the negative selectable marker

CodA, with one that could also act on the one newly inserted marker, i.e. D-amino acid oxidase, for negative selection.

However, both of the options for redesigning the construct as suggested by the Examiner would change the basic principle under which the Signer construct was designed to operate. Assuming *arguendo* that the NPT gene was eliminated and only *CodA* remained in the construct (option 2) or that a single transgene was in the construct (option 1), the selection media taught by Signer would not operate for its intended purpose since these are designed to each act on separate different selectable markers with only one designed to act with the *CodA* gene and none designed to act on a single selectable marker as both a positive selection media and a negative selection media.

Thus, contrary to the Examiner's assertion, the modification suggested does indeed require a substantial reconstruction of the specific methods steps of Signer which the Examiner relied on and as such the teachings of the references are not sufficient to render the claims *prima facie* obvious.

Furthermore, neither Signer nor Nasholm provide any teaching or support for the proposed modifications or for any substitutions that would be required to effectuate the modification to Signer proposed by the Examiner. The Examiner attempts to bridge the gap with the other references cited. However, these likewise do not provide the requisite teachings or support as explained below.

ii) Signer modified by Nasholm plus Stougaard and Boeke.

Stougaard discloses that *CodA* can be used as either a negative selectable marker or a positive selectable marker. Nothing in Stougaard discloses the use of *CodA* as a dual-functional selection marker in one construct. Moreover, Nasholm does not teach or disclose the use of D-amino acid oxidase as a dual-functional selection marker for eliminating marker sequences from transgenic plants. Nothing in Signer discloses that one transgene rather than two separate selectable marker genes can be used for marker excision in plants.

The Examiner thus relies on yet another reference, Boeke, for generally teaching "the idea of using one marker for both positive and negative selection" as support for the suggested

modification. (see Final Office Action dated December 2, 2009, p. 12, ll. 11-13, p. 15, ll. 19-20, p. 16, ll. 5-7).

However, this reliance on Boeke is misplaced since it ignores the teaching of the reference as well as the features of the claimed invention. As explained above, the yeast system of Boeke does not teach positive and negative selection using two selection media comprising compounds that act on one selectable marker. Boeke only discloses one selection medium comprising one compound (*i.e.* 5-FOA) which can act on a selectable marker.

In contrast, the method for producing transgenic plants as claimed relates to the use of at least two selection compounds, *i.e.* compounds X and M, which act with one selectable marker (D-amino acid oxidase). Compound X is phytotoxic against plant cells not expressing D-amino acid oxidase, but can be metabolized by the cell expressing D-amino acid oxidase into a non-phytotoxic or less phytotoxic compound. Compound M is non-phytotoxic or moderately phytotoxic against plant cells not expressing D-amino acid oxidase, but can be metabolized by the D-amino acid oxidase into a phytotoxic or more phytotoxic compound.

Even with the knowledge from yeast systems and knowledge that *CodA* or D-amino acids oxidase could be used as either a positive or negative selectable marker, the specific method steps relied on by the Examiner taught by Signer still leads one of skill in the art to use **two** distinct selectable markers with each of the **two** selection media acting on separate distinct selectable markers in plants.

Nothing in the combined teachings of the references leads one skilled in the art to modify the method of Signer to eliminate one of the selectable markers, to only use the other even though it was known as a marker that could be used as either a positive or a negative selectable marker, then to substitute this marker with another marker, and in addition to change the premise of the selection media. Rather the combined teaching of the references leads one skilled in the art to use two distinct selectable markers for marker excision in plants. Moreover, if the *CodA* marker of Signer, known to be either a positive or a negative selectable marker, was substituted with the D-amino acid oxidase of Nasholm, the specific method steps taught by Signer relied upon by the Examiner would still require two selectable markers.

Contrary to the Examiner's assertion, nothing in the combined teachings of the references leads one skilled in the art to eliminate or alter the specific method steps relied upon by the Examiner. See *In re Dow Chem. Co. v. American Cyanamid Co.*, 837 F.2d 469, 473, 5 U.S.P.Q.2d 1529, 1531-32 (Fed. Cir. 1988) ("There must be a reason or suggestion in the art for selecting the procedure used, other than the knowledge learned from the applicant's disclosure.").

It is only from the teaching of the present specification that one would be guided to use a single transgene as a dual functional marker in one construct for marker excision in plants with compounds that act on the selectable marker which is tantamount to impermissible hindsight, which the Court in *KSR* guards against. *KSR*, 127 S. Ct. at 1741 (warning against a "temptation to read into the prior art the teachings of the invention in issue" and instructing courts to "guard against slipping into the use of hindsight."). It is improper to "[use] that which the inventor taught against its teacher." *In re Lee*, 277 F.3d at 1343, citing *W.L. Gore v. Garlock, Inc.*, 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983).

The Examiner concludes that "[t]he convenience of using one marker instead of two is ample motivation for one of ordinary skill in the art to combine the teachings." (Final Office Action dated December 2, 2009, p. 14, ll. 7-8). However, as explained above, this assertion is erroneous since neither Boeke nor any of the other references teaches or suggests the use of one marker on which two compounds can act for positive and negative selection.

The Examiner further concludes that "*KSR* forecloses the argument that a specific teaching, suggestion, or motivation is required to support a finding of obviousness." (Final Office Action dated December 2, 2009, p. 14, ll. 8-11).

However, the *KSR* decision did not totally reject the use of the teaching, suggestion, motivation test as a factor in the obviousness analysis. As explained by the Board in *Ex parte Whelan*, "[t]he Court did not, however, discard the TSM test completely; it noted that its precedents show that an invention 'composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art.'" *Ex parte Whelan*, 89 U.S.P.Q.2d 1078, 1084 (BPAI 2008).

The Board in *Ex parte Whelan*, which reversed the obviousness rejection, further explained the obviousness analysis as follows:

The obviousness rationale addressed in *KSR* was premised on combining elements known in the prior art. *Id.* at 1738-39. A parallel analysis applies, however, to a rejection premised on the obviousness of modifying a known composition to change its properties.

The *KSR* Court noted that obviousness cannot be proven merely by showing that the elements of a claimed device were known in the prior art; it must be shown that those of ordinary skill in the art would have had some “apparent reason to combine the known elements in the fashion claimed.” *Id.* at 1741.

In the same way, when the prior art teaches away from the claimed solution as presented here (FF12, FF20, FF22 and FF 24), obviousness cannot be proven merely by showing that a known composition could have been modified by routine experimentation or solely on the expectation of success; it must be shown that those of ordinary skill in the art would have had some apparent reason to modify the known composition in a way that would result in the claimed composition.

Ex parte Whelan, 89 U.S.P.Q.2d 1078, 1084 (BPAI 2008).

Analogous to the holding in *Ex parte Whelan*, the fact that the construct and method of Signer can be modified and the reliance on the gist or general ideas from the references does not provide the requisite rationale that would have prompted one skill in the art “to modify the known composition in a way that would result in the claimed composition,” especially since the premise relied as the basis for the rejection is not even taught in the Boeke reference. *Id.* (emphasis added). Furthermore, “knowledge of the goal does not render its achievement obvious.” *Abbott Labs. v. Sandoz, Inc.*, 544 F.3d 1341, 1352 (Fed. Cir. 2008).

c) Even With the Suggested Modification, One Skilled In The Art Would Not Arrive At The Claimed Invention.

Assuming *arguendo* that Signer and Nasholm were combinable, the substitution of D-amino acid oxidase into the method of Signer would result in a different method than that claimed. As explained above, the construct and method of Signer use two different selectable markers, an NPT gene and the *CodA* gene. The Examiner alleges that Stougaard teaches that the *CodA* gene can be used as either a positive or a negative selection marker and that Nasholm teaches that D-amino acid oxidase can be used as a positive or a negative selection marker. Substituting these two potential dual-functional marker genes, one skilled in the art would still

not arrive at the claim invention, since the resulting construct would still comprise two different selectable markers, the NPT gene and the dual-functional selectable marker.

Assuming *arguendo* that there was a reason to eliminate the positive selectable marker from the construct of Signer or that there was a reason to substitute a construct comprising two selectable markers with a construct with one dual-functional selectable marker and these modifications were made, one skilled in the art would still not arrive at the claim invention, since the two different selection media would still each act on separate different selectable markers with only one able to act with the *CodA* gene and none of the two different selection media able to act on a single selectable marker, *i.e.* as a positive selection media and as a negative selection media on the same selectable marker.

Because the modification suggested by the Examiner does not arrive at the claimed method, a *prima facie* case of obviousness has not been established for this additional reason.

d) The References Direct One Skilled In the Art In A Different Direction Than The Claimed Method.

It is well established that under 35 U.S.C. § 103 the Examiner must consider the reference as a whole, including portions that teach away from the claimed invention. *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984); see also *KSR Int'l Co. v. Teleflex, Inc.*, 127 S. Ct. 1727, 1740 (2007); MPEP § 2141.03 (VI).

When considered as a whole, Signer directs one skilled in the art in a different direction from the claimed method. For the production of marker-free plants, Signer requires the use of at least two distinct markers, one used as a positive selectable marker (an NPT gene), and the other as the negative selectable marker (the *CodA* gene) even with the knowledge that *CodA* can be used as either a positive or a negative selection marker from the teaching of Stougaard.

During prosecution, the Examiner attempted to dismiss the negative teaching of Signer by asserting:

“[o]ne of ordinary skill in the art would have appreciated the convenience of utilizing one marker instead of two. The fact that Signer et al did not utilize *CodA* for both positive and negative selection does not teach away from the

claimed invention, because the claimed invention is not directed to the use of *CodA*; it is directed to the use of D-amino acid oxidase.” Final Office Action dated December 2, 2009 (pp. 13-14).

The Examiner’s argument is a non sequitur. The use of *CodA* is relevant since this is what the references teach to one skilled in the art and this is what the Examiner is suggesting to modify. Appellants agree that the claims are directed to the use of D-amino acids, however, none of the references point to using **D-amino acids as claimed**. Moreover, the Examiner is grafting the claimed invention into the prior art. *KSR* prohibits such an approach. *KSR International Co. v. Teleflex Inc.*, 127 S. Ct. 1727, 1741 (2007) (warning against a “temptation to read into the prior art the teachings of the invention in issue” and instructing courts to “guard against slipping into the use of hindsight.”).

Stougaard also does not support the Examiner’s argument. It discloses that *CodA* can be used as either a negative selectable marker or a positive selectable marker. It does not teach use of *CodA* as a dual-functional marker in one construct. The Examiner attempts to bridge this gap with an unsupported supposition. The Examiner’s primary reference Signer refutes that supposition. Signer teaches that two distinct selectable markers are required even if one of these is a potential dual-functional marker.

The Examiner argues that “[t]he fact that Signer et al used two markers instead of one does not take away from the teachings of Boeke et al that demonstrate the convenience of using one marker.” (Final Office Action dated December 2, 2009, p. 14). However, the yeast system of Boeke does not teach the use of one selectable marker with two types of selection media comprising compounds which can act on the selectable marker for positive and negative selection. Rather Boeke teaches an initial phenotypic selection which simply starves the cells by not giving one of the necessary elements for growth. This does not correspond to a selection media comprising a compound which acts on the selectable marker. Boeke does not teach a compound which is toxic to the wild-type cells and which is metabolized to a non-toxic compound by the marker. Rather Boeke only teaches a single selection where a nontoxic compound is converted to one that is toxic (Boeke, p. 164, first paragraph). As explained above, the yeast system of Boeke does not teach positive and negative selection using two selection media comprising compounds that act on one selectable marker. Boeke only discloses one selection medium comprising one compound (*i.e.* 5-FOA) which can act on a selectable marker.

Because Boeke does not teach one transgene being used as a dual-functional selectable marker, the entire obviousness argument falls apart.

Thus, contrary to the claimed method, the combined teaching of the references direct one skilled in the art to use two different types of selectable markers and two different selection media comprising compounds where each of the compounds acts with a different selectable marker.

C. Dependent Claim 10 Would Not Have Been Obvious For The Further Reason That The Second Expression Cassette Is Not Localized Between The Sequences Which Allow For Specific Deletion Of The First Expression Cassette.

Individual consideration of claim 10, set forth in Appendix A, in addition to the above arguments is requested. Claim 10 recites that the first expression cassette which comprises the nucleic acid sequence encoding a D-amino acid oxidase is flanked by sequences which allow for specific deletion of the first expression cassette. Additionally, claim 10 recites that the second expression cassette, which is suitable for conferring an agronomically valuable trait, is not localized between the sequences which allow for the specific deletion of the first expression cassette.

The construct taught in Signer, in contrast, comprises direct repeats of the gene of interest which flank the two different types of selectable markers, depicted by the general formula: GI-PS-NS-GI or GI-NS-PS-GI, where GI is the gene of interest, PS is the positive selectable marker gene, and NS is the negative selectable marker gene. (Signer, p. 2, ¶¶ [0006]-[0007]). Signer also teaches that it is the homologous recombination between the direct repeats of the gene of interest which leads to the elimination of the intervening DNA (*i.e.* the DNA between the direct repeats of the gene of interest), leaving behind a construct with only a single copy of the gene of interest itself (Signer, pp. 2, 5, 13; Examples 1-3). Signer teaches that direct repeats of the gene of interest are required in that particular configuration in order to allow for homologous recombination, crossing over, and the elimination of the selectable markers (Signer, pp. 2, 5, 13; Examples 1-3). Thus, in contrast to the construct of claim 10, in the Signer construct the direct repeats of the gene of interest themselves correspond to the sequences which allow for the elimination of the selectable markers.

Because Signer does not teach or suggest a second expression cassette, which is suitable

for conferring an agronomically valuable trait, not being localized between the sequences which allow for the specific deletion of the first expression cassette, a *prima facie* case of obviousness has not been established for claim 10. Reversal of the rejection is respectfully urged.

D. Dependent Claims 3 and 27 Would Not Have Been Obvious For The Further Reason That A Step of Inducing Deletion Is Specified

Individual consideration of claims 3 and 27, set forth in Appendix A, in addition to the above arguments is requested. Claims 3 and 27 also recite that a second expression cassette, which is suitable for conferring an agronomically valuable trait, is not localized between the sequences which allow for the specific deletion of the first expression cassette as with claim 10. Thus, the same arguments above as applied to claim 10 are equally applicable to claims 3 and 27.

Additionally, claims 3 and 27 further specify a step of inducing deletion of the first expression cassette. In contrast, the method of Signer does not induce deletion of the selectable markers but rather waits for the recombination to occur naturally and by chance before conducting the second selection, for example through meiosis (see Signer, for example, p.2 ¶ [0008], p. 12, ¶¶ [0038]-[0039], Example 1).

Because Signer does not teach or suggest a second expression cassette not being localized between the sequences which allow for the specific deletion of the first expression cassette and does not teach or suggest inducing deletion, a *prima facie* case of obviousness has also not been established for claims 3 and 27. Reversal of the rejection is respectfully urged.

VIII. CLAIMS

A copy of the claims involved in the present appeal is attached hereto as Appendix A. As indicated above, the claims in Appendix A include the amendments filed by Applicants on March 8, 2010.

IX. EVIDENCE

No evidence submitted pursuant to 37 C.F.R. §§ 1.130, 1.131, or 1.132 is being relied upon for this appeal. Accordingly, no such evidence is provided in APPENDIX B.

X. RELATED PROCEEDINGS

As stated in section II, *supra*, no related proceedings have been or are now pending. Accordingly, no related decisions are provided in APPENDIX C.

XI. CONCLUSION

In sum, for the reasons discussed above, reversal of the obviousness rejections under 35 U.S.C. § 103(a) of claims 1-4, 10, and 27 is strongly urged.

This Appeal Brief is filed within the two month period from the date of filing the Notice of Appeal to and including June 1, 2010 pursuant to 37 CFR § 41.37(a).

The requisite fee for filing an appeal brief is paid by credit card. If any additional fee is due, please charge our Deposit Account No. 03-2775, under Order No. 13987-00022-US from which the undersigned is authorized to draw.

Dated: May 31, 2010

Respectfully submitted,

By 
Roberte M. D. Makowski, Ph.D.

Registration No.: 55,421
CONNOLLY BOVE LODGE & HUTZ LLP
1007 North Orange Street
P. O. Box 2207
Wilmington, Delaware 19899-2207
(302) 658-9141
(302) 658-5614 (Fax)
Attorney for Appellants

APPENDIX A**Claims Involved in the Appeal of Application Serial No. 10/593,181**

1. (Previously presented) A method for producing a transgenic plant comprising:
 - i) transforming a plant cell with a first expression cassette comprising a nucleic acid sequence encoding a D-amino acid oxidase operably linked with a promoter allowing expression in plant cells or plants, in combination with at least one second expression cassette suitable for conferring to said plant an agronomically valuable trait, and
 - ii) providing at least one first compound X, which is phytotoxic against plant cells not functionally expressing said D-amino acid oxidase, wherein said compound X can be metabolized by said D-amino acid oxidase into one or more compound(s) Y which are non-phytotoxic or less phytotoxic than compound X, and
 - iii) treating said transformed plant cells of step i) with said first compound X in a phytotoxic concentration and selecting plant cells comprising in their genome both said first and said second expression cassette, wherein said first expression cassette is conferring resistance to said transformed plant cells against said compound X by expression of said D-amino acid oxidase, and
 - iv) providing at least one second compound M, which is non-phytotoxic or moderately phytotoxic against plant cells not functionally expressing said D-amino acid oxidase, wherein said compound M can be metabolized by said D-amino acid oxidase into one or more compound(s) N which are phytotoxic or more phytotoxic than compound M, and
 - v) breaking the combination between said first expression cassette and said second expression cassette and treating resulting said plant cells with said second compound M in a concentration toxic to plant cells still comprising said first expression cassette, and selecting plant cells comprising said second expression cassette but lacking said first expression cassette,wherein said first compound X and said second compound M both comprise a D-amino acid.

2. (Previously presented) The method of Claim 1, wherein said first expression cassette for said D-amino acid oxidase and said second expression cassette for said agronomically valuable trait are both comprised in one DNA construct and combination is broken by deletion or excision of said first expression cassette for said D-amino acid oxidase.

3. (Previously presented) The method of Claim 1, wherein said method for producing a transgenic plant comprises the steps of:

- i) transforming a plant cell with a first DNA construct comprising
 - a) a first expression cassette comprising a nucleic acid sequence encoding a D-amino acid oxidase operably linked with a promoter allowing expression in plant cells or plants, wherein said first expression cassette is flanked by sequences which allow for specific deletion of said first expression cassette, and
 - b) at least one second expression cassette suitable for conferring to said plant an agronomically valuable trait, wherein said second expression cassette is not localized between said sequences which allow for specific deletion of said first expression cassette, and
- ii) providing at least one first compound X, which is phytotoxic against plant cells not functionally expressing said D-amino acid oxidase, wherein said compound X can be metabolized by said D-amino acid oxidase into one or more compound(s) Y which are non-phytotoxic or less phytotoxic than compound X, and
- iii) treating said transformed plant cells of step i) with said first compound X in a phytotoxic concentration and selecting plant cells comprising in their genome said first DNA construct, conferring resistance to said transformed plant cells against said compound X by expression of said D-amino acid oxidase, and
- iv) providing at least one second compound M, which is non-phytotoxic or moderately phytotoxic against plant cells not functionally expressing said D-amino acid oxidase, wherein said compound M can be metabolized by said D-amino acid oxidase into one or more compound(s) N which are phytotoxic or more phytotoxic than compound M, and

- v) inducing deletion of said first expression cassette from the genome of said transformed plant cells and treating said plant cells with said second compound M in a concentration toxic to plant cells still comprising said first expression cassette, thereby selecting plant cells comprising said second expression cassette but lacking said first expression cassette.
4. (Previously presented) The method of claim 1 further comprising the step of regeneration of a fertile plant.
5. (Withdrawn) The method of claim 1, wherein said first compound X comprises a D-amino acid selected from the group consisting of D-tryptophane, D-histidine, D-arginine, D-threonine, D-methionine, D-serine, and D-alanine.
6. (Withdrawn) The method of claim 1, wherein said second compound M comprises a D-amino acid selected from the group consisting of D-isoleucine, D-valine, D-asparagine, D-leucine, D-lysine, D-proline, and D-glutamine.
7. (Withdrawn) The method of claim 1, wherein deletion of said first expression cassette for the D-amino acid oxidase is realized by a method selected from:
- a) recombination induced by a sequence specific recombinase, wherein said first expression cassette is flanked by corresponding recombination sites in a way that recombination between said flanking recombination sites results in deletion of the sequences in-between from the genome, or
 - b) homologous recombination between homology sequences A and A' flanking said first expression cassette, induced by a sequence-specific double-strand break caused by a sequence specific endonuclease, wherein said homology sequences A and A' have sufficient length and homology in order to ensure homologous recombination between A and A', and having an orientation which – upon recombination between A and A' – will lead to excision of said first expression cassette from the genome of said plant.

8. (Withdrawn) The method of Claim 7, wherein the recombinase or sequence-specific endonuclease, respectively, is expressed or combined with its corresponding recombination or recognition site, respectively, by a method selected from the group consisting of:

- a) incorporation of a second expression cassette for expression of the recombinase or sequence-specific endonuclease operably linked to a plant promoter into said DNA construct, together with said first expression cassette flanked by said sequences which allow for specific deletion,
- b) incorporation of a second expression cassette for expression of the recombinase or sequence-specific endonuclease operably linked to a plant promoter into the plant cells or plants used as target material for the transformation thereby generating master cell lines or cells,
- c) incorporation of a second expression cassette for expression of the recombinase or sequence-specific endonuclease operably linked to a plant promoter into a separate DNA construct, which is transformed by way of co-transformation with said first DNA construct into said plant cells, and
- d) incorporation of a second expression cassette for expression of the recombinase or sequence-specific endonuclease operably linked to a plant promoter into the plant cells or plants which are subsequently crossed with plants comprising the DNA construct of the invention.

9. (Withdrawn) The method of Claim 7, wherein deletion of said first expression cassette for the D-amino acid oxidase is induced or activated by inducing expression and/or activity of said sequence-specific recombinase or endonuclease by a method selected from the group consisting of

- a) inducible expression by operably linking the sequence encoding said recombinase or endonuclease to an inducible promoter, and
- b) inducible activation, by employing a modified recombinase or endonuclease comprising a ligand-binding-domain, wherein activity of said modified recombinase or endonuclease can be modified by treatment of a compound having binding activity to said ligand-binding-domain.

10. (Previously presented) The method of claim 2, wherein the DNA construct comprises
- a) a first expression cassette comprising a nucleic acid sequence encoding a D-amino acid oxidase operably linked with a promoter allowing expression in plant cells or plants, wherein said first expression cassette is flanked by sequences which allow for specific deletion of said first expression cassette, and
 - b) at least one second expression cassette suitable for conferring to said plant an agronomically valuable trait, wherein said second expression cassette is not localized between said sequences which allow for specific deletion of said first expression cassette

and the resulting plant cell or plant is selection marker free.

11. (Withdrawn) A DNA construct suitable for the method of claim 1, comprising
- a) a first expression cassette comprising a nucleic acid sequence encoding a D-amino acid oxidase operably linked with a promoter allowing expression in plant cells or plants, wherein said first expression cassette is flanked by sequences which allow for specific deletion of said first expression cassette, and
 - b) at least one second expression cassette suitable for conferring to said plant an agronomically valuable trait, wherein said second expression cassette is not localized between said sequences which allow for specific deletion of said first expression cassette.

12. (Withdrawn) The DNA construct of Claim 11, wherein said D-amino acid oxidase expressed from said first expression cassette has metabolizing activity against at least one D-amino acid and comprises the following consensus sequence:

[LIVM]-[LIVM]-H*-[NHA]-Y-G-x-[GSA]-[GSA]-x-G-x₅-G-x-A

wherein amino acid residues given in brackets represent alternative residues for the respective position, x represents any amino acid residue, and indices numbers indicate the respective number of consecutive amino acid residues.

13. (Withdrawn) The DNA construct of Claim 11, wherein said D-amino acid oxidase has enzymatic activity against at least one of the amino acids selected from the group consisting of D-alanine, D-serine, D-isoleucine, D-valine.

14. (Withdrawn) The DNA construct of claim 11 wherein said D-amino acid oxidase is described by a sequence of the group consisting of sequences described by GenBank or SwisProt Acc.No. JX0152, O01739, O33145, O35078, O45307, P00371, P14920, P18894, P22942, P24552, P31228, P80324, Q19564, Q28382, Q7PWX4, Q7PWY8, Q7Q7G4, Q7SFW4, Q7Z312, Q82MI8, Q86JV2, Q8N552, Q8P4M9, Q8PG95, Q8R2R2, Q8SZN5, Q8VCW7, Q921M5, Q922Z0, Q95XG9, Q99042, Q99489, Q9C1L2, Q9JXF8, Q9V5P1, Q9VM80, Q9X7P6, Q9Y7N4, Q9Z1M5, Q9Z302, and U60066.

15. (Withdrawn) The DNA construct of claim 11 wherein said D-amino acid oxidase is selected from the group of amino acid sequences consisting of

- a) sequences described by SEQ ID NO: 2, 4, 6, 8, 10, 12, and 14,
- b) sequences having a sequence homology of at least 40% with a sequence as described by SEQ ID NO: 2, 4, 6, 8, 10, 12, and 14, and
- c) sequences hybridizing under low or high stringency conditions with a sequence as described by SEQ ID NO: 2, 4, 6, 8, 10, 12, and 14.

16. (Withdrawn) The DNA construct of claim 11, wherein said sequences which allow for specific deletion of said first expression cassette are selected from the group of sequences consisting of

- a) recombination sites for a sequences-specific recombinase arranged in a way that recombination between said flanking recombination sites results in deletion of the sequences in-between from the genome, and
- b) homology sequences A and A' having a sufficient length and homology in order to ensure homologous recombination between A and A', and having an orientation which – upon recombination between A and A' – will result in deletion of the sequences in-between from the genome.

17. (Withdrawn) The DNA construct of Claim 16, wherein said recombination sites correspond to a recombinase selected from the group consisting of a cre recombinase, a FLP recombinase, a Gin recombinase, a Pin recombinase, and a R recombinase.
18. (Withdrawn) The DNA construct of Claim 16, wherein said DNA construct comprises a recognition site of at least 10 base pairs for a sequence specific endonuclease between said homology sequences A and A'.
19. (Withdrawn) The DNA construct of Claim 18, wherein said recognition site corresponds to a sequence-specific endonuclease selected from the group consisting of homing endonucleases I-SceI, I-CpaI, I-CpaII, I-CreI, and I-ChuI and chimeras thereof with ligand-binding domains.
20. (Withdrawn) The DNA construct of claim 16, wherein said DNA construct further comprises a expression cassette for the sequence specific endonuclease or recombinase suitable for mediating deletion of the first expression cassette for the D-amino acid oxidase.
21. (Withdrawn) The DNA construct of Claim 20, wherein expression and/or activity of said sequence-specific recombinase or endonuclease can be induced and/or activated by a method selected from the group consisting of
- a) inducible expression by operably linking the sequence encoding said recombinase or endonuclease to an inducible promoter, and
 - b) inducible activation, by employing a modified recombinase or endonuclease comprising a ligand-binding-domain, wherein activity of said modified recombinase or endonuclease can be modified by treatment of a compound having binding activity to said ligand-binding-domain.
22. (Withdrawn) A transgenic vector comprising the DNA construct of claim 11.
23. (Withdrawn) A transgenic cell comprising the DNA construct of claim 11 or a vector comprising said construct.

24. (Withdrawn) The transgenic cell of Claim 23, wherein said cell is a plant cell.
25. (Withdrawn) A transgenic, non-human organism comprising the DNA construct of claim 11, a vector comprising said construct, or a transgenic cell comprising said construct or vector.
26. (Withdrawn) The transgenic, non-human organism of Claim 25 wherein said organism is a plant.
27. (Previously presented) The method of claim 3 further comprising the step of regeneration of a fertile plant.
28. (Withdrawn) The method of claim 3, wherein said first compound X comprises a D-amino acid selected from the group consisting of D-tryptophane, D-histidine, D-arginine, D-threonine, D-methionine, D-serine, and D-alanine.
29. (Withdrawn) The method of claim 3, wherein said second compound M comprises a D-amino acid selected from the group consisting of D-isoleucine, D-valine, D-asparagine, D-leucine, D-lysine, D-proline, and D-glutamine.
30. (Withdrawn) The method of Claim 1, wherein said first expression cassette for said D-amino acid oxidase and said second expression cassette for said agronomically valuable trait are comprised on separate DNA constructs which are transformed in combination by co-transformation into said plant cells, and the combination is broken by subsequent segregation of the two expression cassettes.

APPENDIX B

None.

APPENDIX C

None.